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Trehalose for use in exercise

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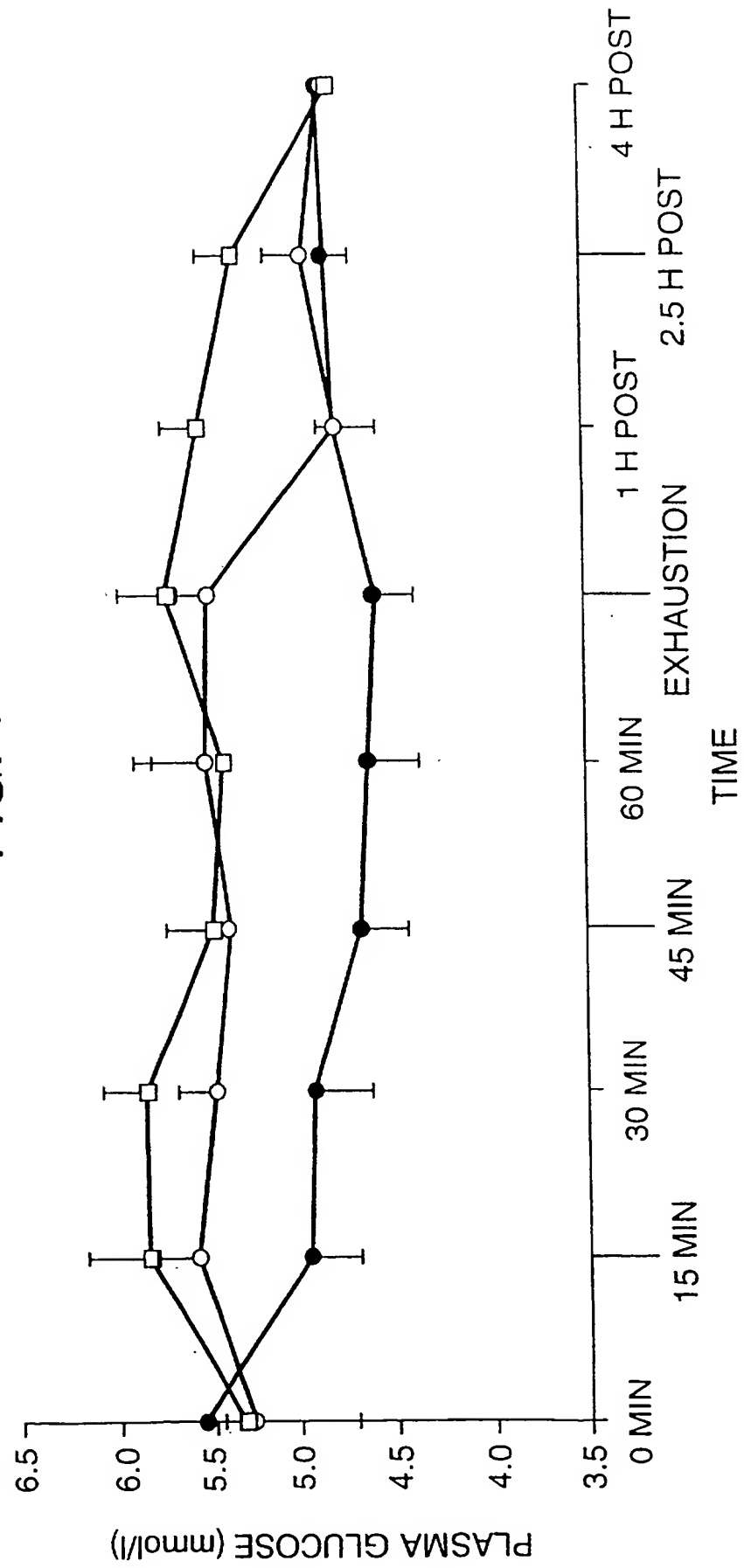
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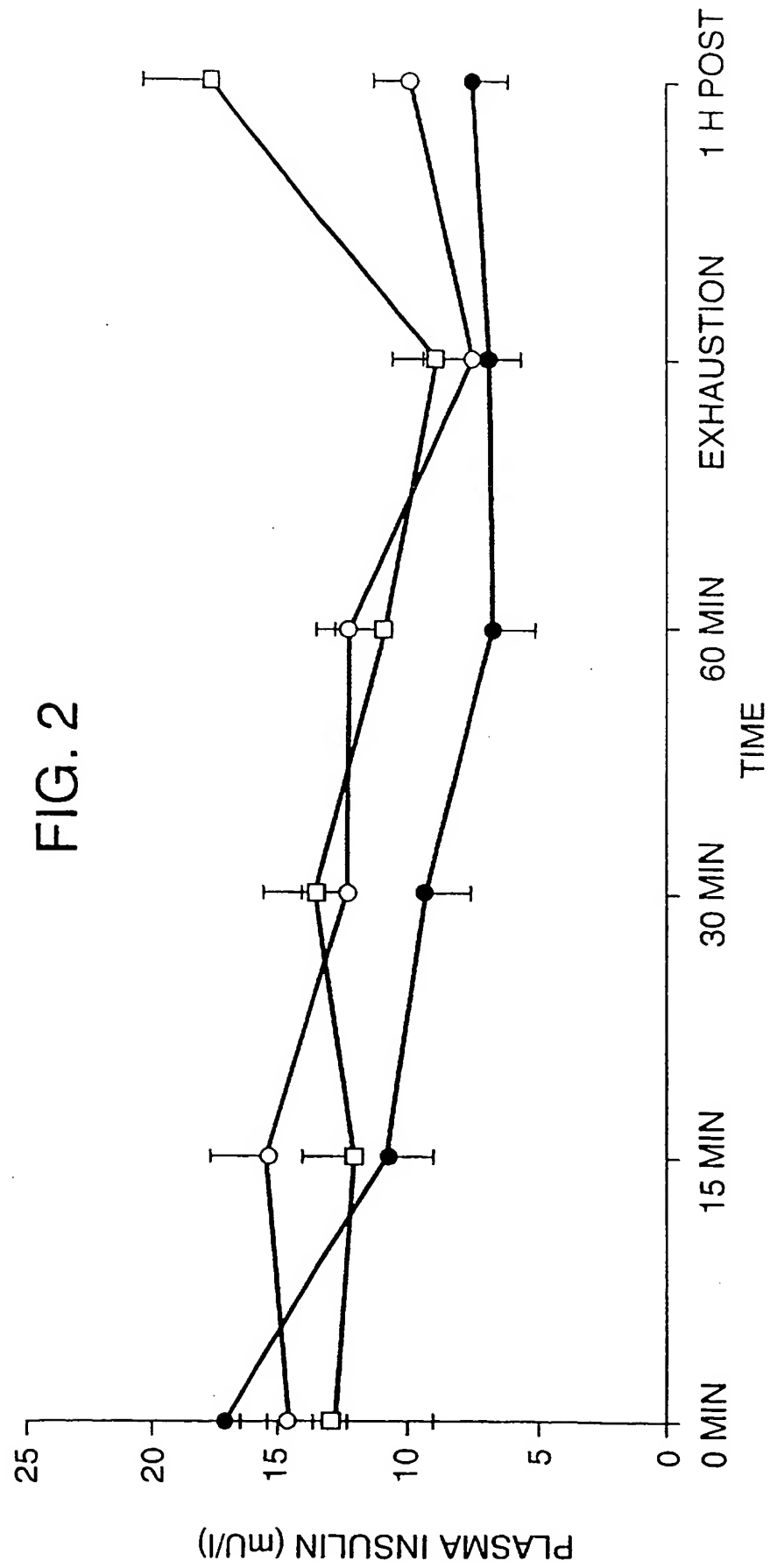
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(54) Abstract Title
Trehalose for use in exercise

(57) The invention provides the use of trehalose for the preparation of a nutritional composition for oral administration to a subject during or shortly before physical exercise to maintain the blood glucose level of the subject for a sustained period during and after said exercise. Preferably, the nutritional composition is a sports drink, however it may be other foodstuffs such as confectioneries, biscuits, cakes, etc. When the composition is a drink, it may be provided as a concentrate to be diluted with water and is preferably isotonic or hypotonic. The composition preferably comprises at least one salt to enhance uptake through the gastro-intestinal tract.

FIG. 1





USE OF SUGAR COMPOSITIONS

The present invention relates to the use of trehalose for the preparation of nutritional compositions for consumption during or shortly before physical exercise.

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It is now well established that the consumption of carbohydrate containing beverages is beneficial for endurance exercise performance. It has become a common practice in many sports for the participants to ingest sports drinks during both competition and training, for example in cycling, triathlon, marathon, football
10 or rugby. It is also known for participants in recreational pursuits such as walking and disco dancing to consume carbohydrate containing beverages to improve endurance performance. The beverages are also consumed to improve mental alertness during such activities.

15

Both the provision of fluid to replace sweat losses and the provision of an energy substrate, usually in the form of glucose and/or glucose polymers, are important considerations in the formulation of sports drinks. Fatigue during prolonged exercise is associated with both dehydration and depletion of endogenous carbohydrate reserves and low blood glucose concentration. Low
20 blood glucose levels also impair mental concentration and motor skill.

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Most drinks for use during exercise are isotonic. That is to say, they have a osmolality close to that of blood plasma, which is about 290 mmol/kg. Isotonic drinks are rapidly emptied from the stomach and absorbed in the intestine.
Increasing the osmolality of the drinks by adding more glucose slows the rate of gastric emptying and initially draws fluid from the circulation into the gut, causing an undesirable dehydrating effect. Hence, most sports drinks contain glucose at a concentration of 4-6% w/v, together with a small amount of sodium (10-60 mmol/l, usually as sodium citrate) which optimises the rate of glucose and water
30 absorption. Glucose is cotransported with sodium from the gut lumen into the gut epithelial cells, and water follows by osmosis. The addition of sodium at concentrations of 40-80 mmol/l aids retention of fluid in the body, and a

concentration close to 60 mmol/l has been recommended for drinks designed to promote post-exercise recovery.

5 Trehalose (α -D-glucopyranosyl- α -D-glycopyranoside) is a naturally occurring non-reducing disaccharide found in fungi, certain yeasts, certain drought resistant plants and in the blood of insects. However, it has hitherto contributed an insignificant part of most human diets.

10 G. G. Birch in Process Biochemistry, July 1970, page 9, briefly reviews the role of trehalose in nature. The author notes that trehalose is quite sweet tasting, and if it were to be absorbed slowly or not at all by the body without any ill effect could produce a useful non-fattening dietetic or diabetic sugar.

15 It has subsequently been established that trehalose is, in fact, readily absorbed by the body. The mechanism for absorption is thought to be that trehalase enzyme in the microvilli of the small intestine breaks the trehalose down into its constituent glucose monomers, which are then absorbed through the intestinal wall.

20 WO96/08979 describes isotonic or hypotonic sports beverages containing trehalose. The sports beverages containing trehalose are said to be useful for providing rehydration and nutrition during athletic exercise. The use of trehalose rather than glucose is said to be desirable because of the lower osmotic pressure at a given weight concentration of trehalose (a disaccharide) compared to glucose
25 (a monosaccharide). There is no suggestion that trehalose would be superior to glucose for sustained maintenance of blood glucose levels during and after vigorous exercise.

30 EP-A-0619951 describes energy supplements containing trehalose. Data are presented showing that relatively small orally administered doses of trehalose (apparently less than 0.01 g/kg of body weight) boost blood glucose levels. It was also found that the trehalose gives a slightly smaller, but still substantial, insulin response than the equivalent amount of glucose. There is no mention of

administration of compositions containing trehalose during or shortly before vigorous exercise to maintain blood glucose levels following said exercise. On the contrary, both this reference and WO96/08979 indicate that the main usefulness of trehalose in nutrition is to provide a quick boost to blood glucose levels.

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It has now been found by the present inventors that administration of compositions containing trehalose during or shortly before physical exercise not only provides a rapid boost to blood glucose levels but also sustains the blood glucose level of the subject at near-normal levels for at least one hour following the exercise. This is quite different from the response to orally administered glucose, where a hypoglycaemic dip is observed following vigorous exercise that can give rise to feelings of increased exhaustion and reduced alertness of the subject.

15 Accordingly, the present invention provides the use of trehalose for the preparation of a nutritional composition for oral administration to a subject during or shortly before physical exercise to maintain the blood glucose level of the subject for a sustained period during and after said exercise.

20 Preferably the nutritional composition is selected from the group consisting of chocolate confectionery, sugar confectionery, biscuits, fondants, jellies, jams, sauces, puddings, syrups, soft drinks, sweet or savoury snack foods, cakes and other baked goods, ice cream, energy and cereal bars, alcoholic beverages and combinations thereof. More preferably, the nutritional composition is a soft drink, and most preferably it is a sports drink.

Preferably, the composition used in the present invention comprises at least 10% w/w of trehalose, preferably at least 20% w/w of trehalose, more preferably at least 30% w/w trehalose, and most preferably at least 40% or 50% by weight of trehalose, based on the dry weight of the composition.

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Where the composition is a soft drink, it may be somewhat hypertonic, but it is preferably an isotonic or hypotonic solution. Preferably, the osmolarity of the

soft drink is 400 mOsm or less, more preferably it is 300 mOsm or less, and most preferably it is 250 mOsm or less. The concentration of trehalose in the soft drink is preferably 2 to 25 wt.%, more preferably 5 to 15 wt.%.

- 5 Where the composition is a soft drink, the step of preparation may comprise diluting a solid or liquid concentrate with water or carbonated water.

Preferably, the soft drink also comprises at least one salt in an amount sufficient to enhance uptake of the water through the gastrointestinal tract.

- 10 Preferably, the salt is selected from the group consisting of salts of sodium, potassium, magnesium and calcium. Preferably, the salt is present in an amount of 10-100mmol/l, more preferably 40 to 80 mmol/l.

- In addition to trehalose and salts, the compositions may contain other
15 nutrients. Suitable nutrients include monosaccharides such as fructose, mannose, galactose and glucose, and disaccharides other than trehalose such as sucrose, maltose and lactose. Suitable nutrients further include vitamins, minerals, amino acids, peptides and proteins. Suitable vitamins include vitamin C, the B vitamins, pantothenic acid, thiamin, niacin, niacinamide, riboflavin and biotin. Suitable
20 minerals include iron, zinc, chromium and magnesium. Suitable amino acids include the 20 amino acids utilised by humans.

- The compositions may further include appropriate amounts of colouring, artificial and natural flavours, sweeteners and preservatives. The compositions
25 may further include one or more stimulants such as taurine and caffeine.

- In accordance with the present invention, the step of oral administration preferably comprises administration of at least 0.1 g of trehalose per kg body weight of the person, preferably at least 0.3g/kg and more preferably at least
30 0.5 g/kg.

Preferably, the physical exercise is vigorous exercise, and more preferably the physical exercise is exercise substantially to exhaustion. Suitable forms of exercise include running, football, tennis, basketball, squash, and the like.

5 The present invention is based on the surprising discovery that consumption of trehalose provides a sustained boost to blood glucose levels that lasts for considerably longer than the boost given by an equivalent molar quantity of glucose. In particular, consumption of trehalose seems to maintain the blood glucose level for an extended period even after exercise to exhaustion.

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 The term "maintain" used herein in relation to blood glucose levels signifies that the trehalose provides a blood glucose level that is higher in a statistically significant amount than is observed for a placebo containing an equivalent amount of water and no carbohydrate. Preferably, in the use according to the present
15 invention, the blood glucose level is maintained at a level at least 0.25 mmol/l above the level for a carbohydrate-free placebo of equal liquid volume, and more preferably at least 0.40 mmol/l above that level.

 Preferably, the blood glucose level is maintained for at least 90 minutes
20 following administration, and more preferably for at least 150 minutes after administration. Preferably, the trehalose is administered no more than one hour before the start of exercise, and more preferably no more than 10 minutes before the exercise, and most preferably during the exercise.

25 Preferably, the blood glucose level is maintained for at least one hour following the physical exercise, and more preferably for at least 90 minutes following the physical exercise.

 Specific examples of the present invention will now be described further in
30 the following procedures and examples, with reference to the accompanying drawings, in which:-

Figure 1 shows a graph of blood plasma glucose concentration against time during a cycle ride to exhaustion at 70% VO_2max . Data are shown for subjects given a placebo (filled circles), a 5 wt.% glucose sports drink (open circles) and a 10 wt.% trehalose sports drink (open squares).

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Figure 2 shows graphs of plasma insulin concentration against time measured for the subjects in the experiment of Figure 1.

Procedure 1

10 The effects of a trehalose solution on metabolic responses, perception of effort, and endurance performance during exercise to exhaustion at a constant exercise intensity were assessed as follows.

Nine healthy male human volunteers, aged 21 ± 1 years (mean \pm S.E.M.),
 15 body mass 81.8 ± 2.0 kg, height 1.79 ± 0.02 m, body mass index (BMI) 25.4 ± 0.5 kg/m^2 volunteered as subjects for the experiment and gave written informed consent. All subjects were recreationally active and familiar with exercise sessions lasting 1-3 hours. None of the subjects had suffered an illness in the preceding 3 weeks. None of the subjects had recently modified their dietary
 20 energy intake and none had undergone marked weight changes in the previous 3 months. Subjects were required to abstain from alcohol intake in the 24 hours preceding each experimental trial. All subjects were given a test drink containing 5 g trehalose prior to commencing the main study. None of the subjects reported any gastrointestinal discomfort following the test drink.

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The aerobic fitness of the subjects was quantified by measurement of their maximal oxygen uptake (VO_2max). Maximal oxygen uptake was determined using a continuous incremental protocol on an electrically braked cycle ergometer (Lode Excalibur (Registered Trade Mark), Holland). Following a 3-minute warm-up at
 30 120W, subjects began cycling at an initial work rate of 150W with increments of 30W every 2 minutes until fatigue. During the second minute of each work rate an expired gas sample was collected into a Douglas bag. A paramagnetic oxygen analyser (Servomex 1420B (Registered Trade Mark), Crowborough, UK) and an

infrared carbon dioxide analyser (Servomex 1415B) were used along with a dry gas meter (Harvard Apparatus Ltd, Edenbridge, UK) for determination of minute ventilation, $\dot{V}O_2$ and $\dot{V}CO_2$. The BASES criteria for attainment of $\dot{V}O_{2max}$ were adopted. From the $\dot{V}O_2$ -work rate relationship, the work rate equivalent to 70% $\dot{V}O_{2max}$ was interpolated.

Subjects performed three bouts of exercise on separate occasions at least one week apart. Following an overnight fast, subjects cycled on cycle ergometer at a work rate equivalent to 70% $\dot{V}O_{2max}$ until exhaustion. On each of these occasions the subjects were randomly assigned to one of three experimental treatments: glucose (5% w/v), trehalose (10% w/v) or placebo solutions. Each solution was flavoured with sugar-free lemon cordial and contained 20 mmol/l trisodium citrate. The osmolality of the drinks was measured using a freezing point osmometer (Advanced Instruments) and was 110, 361, and 386 mOsm/kg for the PLA, GLU, and T10 drinks, respectively (note that 80 mOsm/kg of each drink was attributable to the added trisodium citrate). The treatments were blinded to the subjects.

Subjects reported to the laboratory in the morning following an overnight fast and nude body weight was recorded. Subjects were instructed to keep their daily exercise to a minimum during the 72 hours preceding each exercise test and to standardise their diet for the 24 hours prior to each test. A 4 cm, 21 g Venflon (Registered Trade Mark) cannula was inserted into an antecubital vein and an initial resting blood sample was obtained. Immediately after this the subject consumed 5 ml per kg body mass of the prescribed drink and then began cycling at 70% $\dot{V}O_{2max}$ until volitional fatigue. Further blood samples were obtained at 15-minute intervals during exercise and at test cessation. Following each blood sample subjects ingested 2 ml per kg body mass of their prescribed solution. Heart rate was monitored at 15-minute intervals using a radiotelemetric device (Polar Electro, Kempele, Finland) and samples of expired gas were collected into Douglas bags after 35, 70 and 105 minutes of exercise for determination of $\dot{V}O_2$ and respiratory exchange ratio. Whole body rates of carbohydrate oxidation were estimated from the gas exchange measurements. Ratings of perceived exertion

(Borg, 1982) were made at 15-minute intervals. Immediately after the post-exercise blood sample has been obtained, subjects towelled dry and nude body weight was measured. Further blood samples were obtained at 1, 2.5 and 4 hours post-exercise, with the subject in a seated position. The subjects consumed an additional 5 ml per kg body mass of the prescribed drinks immediately following the blood samples taken at 1 and 2.5 hours post-exercise. Room conditions were 19.3 ± 0.3 °C and $58 \pm 2\%$ relative humidity.

Blood was placed into EDTA tubes (4.5 ml) and lithium heparin tubes (7 ml). An aliquot from the EDTA tube was used to determine haemoglobin concentration and haematocrit so that plasma volume changes can be estimated according to Dill and Costill (1974). The remainder was used for analysis of differential white blood cell counts (not reported here). Blood from the heparinised tube was centrifuged at 1500 g for 10 minutes at 4°C to obtain plasma. The latter was stored at -70°C prior to analysis for glucose and lactate (Sigma Chemicals kits), insulin and cortisol (by radioimmunoassay using ICN Biomedicals antibody coated tube kits), and total protein (Biuret method; Sigma Chemicals). The coefficient of variation for the assays was $\pm 2.4\%$ for glucose, $\pm 7.3\%$ for lactate, $\pm 3.4\%$ for protein $\pm 3.5\%$ for insulin and $\pm 2.4\%$ for cortisol.

20

Differences with time and treatment were assessed using a repeated measures ANOVA with Tukey post hoc tests where appropriate. The accepted level of significance was $P < 0.05$. Friedman's non-parametric test was also used where appropriate (e.g. for endurance times and body mass changes). All data in the text, Tables and Figures are reported as mean \pm standard error of the mean (S.E.M.) for eight subjects from whom we obtained the full complement of blood samples.

The mean VO_2max of the subjects was 53.1 ± 1.9 ml/kg/min and the mean work rate equivalent to 70% VO_2max used in the exercise trials was 198 ± 12 W. Oxygen uptakes averaged for the 35 and 70-minute time points during exercise were $78.5 \pm 4.1\%$, $76.6 \pm 3.4\%$ and $72.8 \pm 2.6\%$ VO_2max for the PLA, GLU and T10 trials, respectively (no significant difference between trials, $P > 0.05$). The

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mean total fluid intakes (up to the end of exercise) were 1113 ± 86 ml, 1257 ± 84 ml and 1163 ± 102 ml for the placebo (PLA) 5% w/v glucose (GLU) and 10% w/v trehalose (T10) drinks, respectively. The mean total sugar intakes (up to the end of exercise) were 62.8 ± 4.2 g and 116.3 ± 10.2 g for the glucose and trehalose treatments, respectively.

Time to fatigue was 77 ± 8 , 98 ± 8 and 86 ± 10 minutes on the PLA, GLU and T10 trials, respectively. Performance on the GLU trial was significantly longer than on the PLA trial ($P < 0.05$). Performance on the T10 trial was not significantly different from that on the GLU or PLA trial. Six of the nine subjects cycled for longer on T10 than on PLA, but only two of the subjects cycled for longer on T10 than on GLU. Interestingly, these two subjects had the longest endurance times among this subject group.

From the oxygen uptake and RER data, it was calculated that the rate of carbohydrate oxidation during exercise was similar on all treatments.

Heart rate was similar on all three trials and averaged 165 ± 4 beats/mm after 30 minutes of cycling and 172 ± 5 beats/mm at exhaustion. Perceived exertion was not significantly affected by glucose or trehalose ingestion compared with placebo.

Resting plasma lactate concentration was 0.8 ± 0.1 mmol/l and at exhaustion had increased to 3.5 ± 0.7 , 3.0 ± 0.5 and 3.1 ± 0.4 mmol/l on the PLA, GLU and T10 trials, respectively. There were no significant differences in the plasma lactate response to exercise between trials.

The mean resting plasma glucose concentration before consumption of the drinks was 5.4 mmol/l. The change in plasma glucose concentration during exercise on the different trials is shown in Figure 1. There was a significant Time*Trial interaction ($F_{8,64} = 3.60$; $P < 0.05$) for the plasma glucose responses. On the PLA treatment the plasma glucose concentration fell significantly during exercise and at exhaustion was 4.6 mmol/l. On the GLU and T10 trials, plasma

glucose concentration was maintained at or slightly above pre-exercise levels throughout exercise. At 1 hour and 2.5 hours post-exercise, the plasma glucose concentration on the T10 trial was significantly higher than on GLU or PLA (both $P < 0.05$). This demonstrates the sustained nature of glucose release following
 5 ingestion of the trehalose.

Changes in plasma volume were small but there was a significant fall in plasma volume during the first 15 minutes of exercise on all trials which was gradually restored during the remainder of the exercise bouts. At the end of
 10 exercise, changes in plasma volume were $-6.5 \pm 1.9\%$, $-6.2 \pm 1.8\%$, and $-7.8 \pm 1.4\%$ for the PLA, GLU, and T10 trials, respectively. Body mass changed by a similar amount on all three trials, falling on average by only 0.2 kg. Taking into account the volume of fluid consumed during the trials, sweat losses amounted to 1.4 ± 0.2 litres during exercise.

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There was a main effect of time on the plasma protein concentration ($F_{4,32} = 3.57$; $P < 0.02$) which increased on average by 8% during the exercise trials but there were no significant differences between the treatments.

20 The mean resting plasma insulin concentration before consumption of the drinks was 14 mU/l. The change in plasma insulin concentration during the three exercise trials is shown in Figure 2. There was a significant Time*Trial interaction ($F_{4,32} = 5.27$; $P < 0.05$) for the plasma insulin responses. On the PLA treatment the plasma insulin concentration fell significantly during exercise. Following GLU and
 25 T10 ingestion, plasma insulin concentration was maintained at pre-exercise levels throughout exercise. At 1 hour, 2.5 hours and 4 hours post-exercise the plasma insulin concentration was significantly higher on the T10 trial compared with both the GLU trial ($P < 0.05$) and the PLA trial ($P < 0.01$).

30 At exhaustion, plasma cortisol concentration had increased significantly on all trials compared with pre-exercise, although there were no significant differences between trials.

Example 1

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A sports drink for use in accordance with the present invention is prepared from the following ingredients:

	Trehalose dihydrate	83.3 g
	Acesulfame K	0.03 g
10	Aspartame	0.03 g
	Lemon juice concentrate	25 ml

The ingredients are dissolved in 0.5 litres of water at 25°C, and when fully dissolved the liquid is made up to 1.0 litres with water. The solution is allowed to stand for 30 minutes before use.

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The sports drink will normally be consumed in an amount of 100 to 500 ml during or shortly before vigorous exercise.

The above embodiments have been described by way of example only.

20 Many other embodiments of the present invention as defined in the accompanying claims will be apparent to the skilled reader.

CLAIMS

1. Use of trehalose for the preparation of a nutritional composition for oral administration to a subject during or shortly before physical exercise to maintain the blood glucose level of the subject for a sustained period during and after said exercise.
2. Use according to claim 1, wherein the nutritional composition is selected from the group consisting of chocolate confectionery, sugar confectionery, biscuits, fondants, jellies, jams, sauces, puddings, syrups, soft drinks, sweet or savoury snack foods, cakes and other baked goods, ice cream, and combinations thereof.
3. Use according to claim 2, wherein the nutritional composition is a soft drink.
4. Use according to claim 3, wherein the step of preparation comprises diluting concentrate with water.
5. Use according to claim 3 or 4, wherein the soft drink has an osmolarity of 400 mOsm or less.
6. Use according to claim 3, 4 or 5, wherein the soft drink also comprises at least one salt in an amount sufficient to enhance uptake of the water through the intestinal tract.
7. Use according to claim 6 wherein the salt is selected from the group consisting of salts of sodium, potassium, magnesium and calcium.
8. Use according to any preceding claim, wherein the nutritional composition also comprises amino acid residues.
9. Use according to any preceding claim, wherein the nutritional composition also comprises a stimulant.

10. Use according to claim 9, wherein the stimulant is selected from the group consisting of caffeine and taurine and mixtures thereof.
11. Use according to any preceding claim, wherein the step of oral
5 administration comprises administration of at least 0.1 g of trehalose per kg body weight of the person, preferably at least 0.3g/kg and more preferably at least 0.5 g/kg.
12. Use according to any preceding claim, wherein the composition comprises
10 at least 10% w/w of trehalose, preferably at least 20% w/w of trehalose, more preferably at least 30% w/w trehalose, and most preferably at least 40% or 50% by weight of trehalose, based on the dry weight of the composition.
13. Use according to any preceding claim, wherein the physical exercise is
15 exercise substantially to exhaustion.
14. Use according to any preceding claim, wherein the blood glucose level is maintained at a level at least 0.25 mmol/l above the level for a carbohydrate-free placebo of equal liquid volume.
20
15. Use according to any preceding claim, wherein the blood glucose level is maintained for at least 2 hours following administration.
16. Use according to any preceding claim, wherein the blood glucose level is
25 maintained for at least one hour following the said physical exercise.



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Claims searched: 1-16

Examiner: Anwar Gilani
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Patents Act 1977 Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK CI (Ed.R): A2B (BLC, BMH9)

Int CI (Ed.7): A23L 1/09, 1/30, 2/38, 2/52, 2/60

Other: Online: WPI, EPODOC, JAPIO, CAS-ONLINE, FROSTI, FSTA

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
X	EP0619951 A2 (HAYASHIBARA SEIBUTSU) whole document	1 at least
X	WO 96/08979 A1 (QUADRANT HOLDINGS CAMBRIDGE) whole document	1 at least
X	Abstract of JP 9173017 A (LOTTE) see WPI abstract	1 at least

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.